Environmental Enrichment Promotes Adaptation to Environment Rearrangement in Younger but not Older Adolescent Rats

Dana E. Cobb

Appalachian State University

Abstract

Experiences, such as environmental enrichment (EE), that allow for exploration often lead to brain changes and alter novelty-seeking behaviors, and adolescence is a developmental period in which these behaviors increase. In this study, the effects of EE during adolescence on preference for familiar objects that have been rearranged using an object-in-place (OiP) task and on neural activation in the hippocampus, which is implicated in the detection of novel spatial relationships, was investigated. Adolescent Long-Evans rats (n=16) were exposed to EE between postnatal days (PND) 25 and 48. Age-matched controls (n=16) experienced a non-enriched home-cage. Two-trial OiP testing occurred on PND 36 and 50 with two delays (15 and 60 min) in an open field containing four objects. Time in direct contact with the rearranged objects at each delay trial was measured. After behavioral testing, brain tissue was processed to examine levels of neural activity in the hippocampus. At PND 36, EE rats spent less time than no-EE rats investigating the rearranged objects at the 15 min delay (p<.0519), and more time at these objects at the 60 min delay than no-EE rats (p<.0089). However, at both delays EE rats spent an equal proportion of time investigating both sets of objects. No significant interaction of EE and OiP task delay was seen at PND 50. Histology showed 24.4% fewer active neurons in CA1 of EE rats than no-EE rats (p<.0336), no difference between groups in DG activation. While neural data support the conclusion that EE animals recognize novelty, behavioral results indicate a decrease in novelty preference in younger, but not older adolescent animals, as a result of EE.

Keywords: object-in-place, investigation, novelty preference, exploratory strategies

Environmental Enrichment Promotes Adaptation to Environment Rearrangement in Younger but not Older Adolescent Rats

Exploration allows an animal to experience, learn about, and practice investigating familiar and novel features in an environment through active investigation (Forgays & Forgays, 1952; Lynn & Brown, 2009). Experiences, such as environmental enrichment (EE), that provide opportunity for exploration often lead to brain and behavior changes (Cain, Green, & Bardo, 2006; Forgays & Forgays, 1952). EE can alter brain development, enhance learning and memory, change emotional responses, and affect aspects of exploratory behavior, including novelty preference and investigation strategies (Cain, Green, & Bardo, 2006; Forgays & Forgays, 1952; van Praag, Kempermann, & Gage, 2000). In addition, EE can improve problem solving skills (Forgays & Forgays, 1951; Hymovitch, 1952), reduce frequency of abnormal behavior and stress, and increase the overall well-being of the animal (Würbel, Stauffacher, & von Holst, 1996).

Because EE has been shown to be beneficial to animals in many ways, there has been a recent rise in the promotion of EE in animal facilities as a general policy, in addition to the implementation of enrichment-type programs in schools with the intent of promoting health and decreasing risk-taking behavior, among other goals (Olsson & Dahlborn, 2002). Understanding the effects of EE on risk-taking behavior is perhaps especially relevant in adolescent animals because of the increases in risk-taking behavior influenced by adolescent development (Lynn & Brown, 2009). These behavior changes typically follow a predictable course, and may be influenced by experience, such as EE.

How adolescent animals interact with their environment is a reliable method to measure the outcome of the behavioral changes, such as increases in novelty preference and

exploration, both measures of risk-taking behavior. Typically, novel object preference (NOP; e.g., Barker & Warburton, 2011) and novel location preference (NLP; e.g., Cost et al., 2014) tasks are used to assess novelty preference behaviors. These tasks, however, are much less complex than typical enriched environments are, and, as such, performance on these tasks may not fully elucidate behavior changes in animals that have experienced a complex environment, such as EE, prior to testing.

How EE during adolescence yields brain changes that contribute to changes in exploration strategies and novelty preferences, was investigated using a preference task. The task used is more complex than traditional NOP and NLP tasks, as it has an internal, rearranged environment containing four objects, in addition to external cues.

Adolescence

In the rat, adolescence is considered to be between postnatal day (PND) 21, beginning at weaning, and 60, when early adulthood begins (Candland & Campbell, 1962; Lynn & Brown, 2009; Tirelli, Laviola, & Adrinani, 2003). Numerous, global changes in the brain driven by experience and genetics (including changes in myelination, synaptogenesis, and white matter and grey matter ratio) are connected with behavioral changes in the adolescent animal (Blakemore & Choudury, 2006; Spear, 2000). During this developmental period, wild rats begin venturing out of the nest to forage, with the final intent to leave the nest entirely (Barnea & Nottebohm, 1996; Lynn & Brown, 2009). This increase in exploration is important, as the animals must learn landmarks and the spatial relationships between these landmarks, so as to be able to find food, shelter, and avoid danger reliably (Barnea & Nottebohm, 1996). As a result, adolescence is characterized by an increase in exploratory

behavior, particularly exploration that allows the animal to learn about features of the environment (Lynn & Brown, 2009; Stansfield & Kirstein, 2005).

Exploration can be defined as active investigation, including locomotion, which leads an animal to learn information about its environment (Barker & Warburton, 2009; Bouchon & Will; 1982; Lynn & Brown, 2009). Lynn and Brown (2009) showed that locomotion increased with age, with late adolescent (PND 47-59) rats exploring more than early adolescent (PND 21-33) rats.

Adolescent rats also demonstrate a predisposition towards sensation or novelty seeking, aspects of risk-taking behavior (Blakemore & Choudhury, 2006; Lynn & Brown, 2009; Spear, 2000; Stansfield & Kirstein, 2005). Novelty seeking and risk-taking behavior can be quantified in a laboratory setting by measuring the amount of time an animal spends interacting with novel objects or by measuring latency to approach novel objects, such as in NOP and NLP tasks (e.g., Barker & Warburton, 2011; Cost et al., 2014). Stansfield and Kirstein (2005) demonstrated that adolescent rats spend twice as much time interacting with a novel object as compared to adult rats. Further, the latency to approach the novel object was less for adolescent rats, and they returned to the novel object more frequently as adult rats (Stansfield & Kirstein, 2005). Thus, there is a developmental increase in novelty preference during adolescence, as well (Lynn & Brown, 2009; Stansfield & Kirstein, 2005).

Environmental Enrichment

Environmental enrichment (EE) can also lead to changes in brain and behavior (Ali, Wilson, & Murphey, 2009). Generally, EE in a laboratory setting provides opportunities for complex physical, cognitive, and social stimulation beyond what would be received in standard housing conditions (Bennett, McRae, Levy, & Frick, 2006; Dhanushkodi, Bindu,

Raju, & Kutty, 2007; Meehan & Mench, 2002; Simpson & Kelly, 2011; van Praag, Kempermann, & Gage, 2000). There are two critical features of EE: physical and social. Physical EE involves structural modifications to the environment (e.g., different levels, toys, tunnels, etc.), while social EE involves opportunities to interact with other animals (e.g., housing in cohorts, mixed EE cages, etc.; Meehan & Mench, 2002; Simpson & Kelly; 2011; van Praag et al., 2000). EE is most effective at changing brain circuits and behaviors mediated by those circuits when both of these features are present, perhaps because both features are present in the wild. When both physical and social paradigms are implemented, EE can be used as a model to study experience-dependent brain and behavior changes, as it involves sensory (touch, smell, visual), emotional (stress, fear) and cognitive (novel problem solving) experiences (e.g., Ali, et al., 2009; Lynn & Brown; 2009; Meehan & Mench, 2002).

The effects of EE on the brain are global and include synaptogenesis (Moser, Moser, & Andersen, 1994; Rampon et al., 2000), altered synaptic transmission (Foster & Dumas, 2001; Green, McNaughton,& Barnes, 1990), enhanced neurogenesis (esp. in the hippocampal dentate gyrus), increased cortical thickness, increased glial density, and increased dendritic arborization (e.g., Barnea, Mishal, & Nottebohm, 2006; Diamond, Ingham, Johnson, Bennett, & Rosenzweig, 1976; Kempermann, Kuhn, & Gage, 1997; Kempermann & Gage, 1999; Rosenzweig, 2003; Rosenzweig & Bennett, 1996). The short-term effects of EE include increased neural activation in the cerebral cortex and hippocampus (especially, dentate gyrus and *cornu ammonis* 3) indicating that these regions are involved in the *initial* response to novelty (Ali et al., 2009). Such neural plasticity accompanies the observed changes in behavior.

Like unenriched (no-EE) adolescent animals, adult animals with enriching experiences show a permanent, shorter latency to approach novelty within an environment and a shorter duration of exploration (Cain, Green, & Bardo, 2006; Fernandez-Teruel et al., 2002; Meehan & Mench, 2002). In addition, EE has been shown to improve problem solving skills, performance on memory and learning tasks, and to mitigate age-related deficits of spatial reference memory (Ali et al., 2009; Bennett et al., 2006; Forgays & Forgays, 1951; Hebb, 1947; Simpson & Kelly, 2011; van Praag et al., 2000). EE is also likely to alter these behaviors in adolescent animals. As adolescence is a time during which behaviors related to exploration and novelty-seeking increase, EE during this time could alter these behaviors in a way different from the way it alters these behaviors in adults, which could then be measured in a laboratory setting.

The majority of studies investigating the effects of EE have looked at adult animals' behavior and brain (e.g., Ali et al., 2009; Bennett et al., 2006; Cain, Green, & Bardo, 2006; Forgays & Forgays, 1951; Hebb, 1947; Meehan & Mench, 2002; Simpson & Kelly, 2011; van Praag et al., 2000). Several researchers (e.g., Hymovitch, 1952; Simpson & Kelly, 2011; van Praag et al., 2000) have examined EE across the lifespan and have suggested that there may be a time during which EE is most beneficial, however, when this critical period might be is not specified. It seems likely, however, that this critical period could be during adolescence, as the few studies using adolescent animals indicate that 1) behavioral changes in adolescent animals as a result of EE are different from the changes seen in adult animals, and 2) early EE exposure results in superior problem-solving abilities as compared to exposure to EE later in life (Hymovitch, 1952). Further, adolescence is a time of myriad brain changes (Spear, 2000). If adolescence is a critical period for EE, such biologically

relevant EE could modulate and facilitate both behavior changes and brain changes. Thus, it becomes essential to investigate exactly how EE during adolescence might influence the behaviors seen in these animals, for instance, by using tasks that allow for assessment of novelty preference and risk-taking.

Behavioral Paradigms and Neural Correlates

NOP and NLP tasks are relatively simple tasks that take place in an open field containing two objects (e.g., Barker & Warburton, 2011; Cost et al., 2014). The tasks utilize an acquisition and test phase, between which an object is either replaced or moved to a new location (Barker and Warburton, 2011). Time spent with both objects is recorded. Historically, these tasks have been used to draw conclusions about recognition memory of objects within a field, with an increase in time spent with a novel object, indicating memory for the familiar object. This conclusion has been drawn because animals with hippocampal and perirhinal lesions spend a greater amount of time with the familiar object, and as such it is implied that the animals do not remember the familiar object (e.g., Barker and Warburton, 2009; 2011). However, it might be more reasonable to say that the animal performing the task is displaying a preference for an object (see Moscardo, Salvetti, Becchi, Bertini, & Fabene, 2012). Experience can affect preference, which could be a proxy for memory, and is relevant to the investigation of risk-taking behaviors (Barnea & Nottebohm, 1996; VanElzakker et al., 2008).

Object-in-Place Task. EE during adolescence decreases novelty preference and exploration for same-day delays in NOP and NLP tasks (Cobb & Zrull, 2014). This is in contrast to typical NOP and NLP literature in adult animals, where an increase in novelty preference following EE has been found (e.g., Cain et al., 2006; Fernandez-Teruel et al.,

2002; Meehan & Mench, 2002). In order to determine if this result is a result of age or a result of the task itself, a more complex task paradigm was necessary. This study utilized an object-in-place (OiP) task, first used by Barker and Warburton (2009).The OiP task, which combines elements of traditional NOP and NLP tasks, assesses preference for the identity and location of four objects in an open field during a test phase following a delay (Cost, Lobell, William-Yee, Henderson, & Dohanich, 2014). The OiP task is distinct from NOP and NLP tasks as it requires the integration of information about individual object features within the environment and the contextual relationships between objects and cues external to the environment (Barker & Warburton, 2009,2011; Barker, Bird, Alexander, & Warburton, 2007). In other words, this task requires the animal to encode the features and locations of objects within an environment using external cues.

The delay used for these tasks has been shown to affect performance in adults. Adult, no-EE animals show no discrimination of objects at short delays (5, 30 min; Cost et al., 2014), however at delays longer than 60 min and up to 48 h, a preference for objects is seen (Dix & Aggleton, 1999). Previous studies have indicated that adaptation to novelty following EE does not occur until later delays in adolescent animals in NLP tasks (e.g., Cobb & Zrull, 2014). Therefore, the OiP task was conducted at a short (15 min) and long (60 min) delay to examine effects of delay on task performance. In addition, the OiP task was conducted at two separate ages (PND 36 and 50) to see how preference changes across the adolescent period.

Hippocampus. The OiP task requires the animal to encode features and locations of objects in an environment for later recognition using cues that are both internal and external to the environment. This unique integration requires hippocampal involvement (Barker & Warburton, 2011; Cost et al., 2014).

The hippocampus is involved in many processes, including spatial awareness, which are particularly relevant to the OiP task (Baker & Warburton, 2011; Cost et al., 2014; Dhanushkodi et al., 2007; Nitz & McNaughton, 2004; O'Keefe & Dostrovsky, 1971; O'Keefe & Nadel, 1978; VanElzakker et al., 2008; Will et al., 1986; Bennett, Rosenzeig, Morimoto, & Herbert, 1979). The hippocampus is responsible for incorporating novel spatial information into preexisting representations of a familiar environment, and for the encoding of relationships between elements of the environment (Nitz & McNaughton, 2004; O'Keefe & Dostrovksy, 1971; VanElzakker et al., 2008). This suggests that the hippocampus is essential for OiP memory, the memory of context within an environment.

The dentate gyrus (DG), the input zone of the hippocampus, is important for the detection of novel spatial relationships (VanElzakker et al., 2008; Will et al., 1986). The *cornu ammonis* 1 (CA1), the output zone of the hippocampus, is important for the initial formation of spatial memories, as well as the integration of novelty information from an environment (Cheng & Frank, 2008; VanElzakker et al., 2008). CA1 neurons receive cortical information directly via the entorhinal cortex, and also receive cortical information that has been partially processed by the DG and *cornu ammoni*s 3 (CA3; Figure 1; Andersen, Bliss, & Skrede, 1971; Dhanushkodi et al., 2007; VanElzakker, Fevurly, Breindel, & Spencer, 2008). Thus, CA1 neurons are unique because they receive information from both past and ongoing experiential neural patterns (Dhanuskodi et al., 2007; Lee, Hunsaker, & Kesner, 2005; Meeter, Murre, & Talamini, 2004; VanElzakker et al., 2008). As such, DG and CA1 should be important for novelty recognition tasks that occur at delays, such as the OiP task, and looking at these regions should allow for a determination of if detection and consolidation of novel information is taking place (VanElzakker et al., 2008).

CA1 and DG cells show a divergent response to novelty within an environment (Nitz & McNaughton, 2004). In general, CA1 interneurons exhibit a higher firing rate in the familiar region, and a decreased firing rate in the novel region (Nitz & McNaughton, 2004). In contrast, DG interneurons exhibit a higher firing rate in the novel region, and a decreased firing rate in the familiar region (Nitz & McNaughton, 2004). However, CA1 pyramidal cells increase firing rate in response to novelty, while DG granule cells decrease firing rate in novel regions (Nitz & McNaughton, 2004).

Purpose and Hypotheses

The present study was intended to examine the effects of EE during adolescence on preference for familiar objects that have been rearranged using an OiP task. Adolescence is an important developmental period during which exploratory behaviors and novelty preferences change. These behaviors can be influenced by experiences, such as EE, and the changes tested using the OiP task. In addition, the present study was designed to allow for the examination of neural correlates in the hippocampus.

Given the relationship between EE and performance on tasks assessing novelty preference at different delays seen in previous studies, it was predicted that there would be an interaction between EE and delay for both PND tests (OiP 1 and OiP 2). EE animals received regular EE sessions, while no-EE animals were handled controls. For PND 36 (OiP 1), it was predicted that there would be a difference between groups at the 60 min delay, with EE animals showing a decreased preference for novelty as compared to no-EE animals. Similarly, at the 60 min delay for PND 50 (OiP 2), it was predicted that there would be a decrease in novelty preference of the EE animals, as compared to no-EE animals.

It was predicted that the histological data would support these behavioral predictions for the 60 min delay as well. EE promotes adaptation to the rearrangement of familiar objects in a known environment, such that young animals attend to more familiar features rather than novelty within a known environment. As such, it was expected that EE animals would show an increase in CA1 and a decrease in DG activation, relative to no-EE animals.

Materials and Method

Experimental Design

Behavior was tested using an OiP task, which allows assessment of preference for objects in varied locations in an open field. A split factorial design was used, in which enriched (EE) adolescent male (*n*=8) and female (*n*=8) rats were tested at two delay intervals. No-EE animals (*n*=16, 8 male, 8 female) were tested at the same intervals. Following the behavioral testing, all rats were sacrificed and brain tissue processed to count baseline neuronal activation using c-fos protein as an indicator (see Figure 2).

Subjects

Young Long-Evans rats (*N*=32) were subjects. The rats were provided by Harlan Sprague-Dawley and the Arts & Sciences Animal Facility at Appalachian State University. The rats were housed in pairs or triples in plastic shoebox cages in a temperature and humidity controlled vivarium with a 12 h light-12 h dark illumination cycle. Food and water were supplied *ad libitum*. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Appalachian State University (#15-02, #12-13).

Environmental Enrichment (EE)

Between PND 25 and 48, half of the rats (*n*=16, 8 male, 8 female) were exposed to EE for 1.5 h/day for five of every seven days (Figure 2). The EE paradigm combined aspects

of physical and social EE (Figure 3). Four EE set-ups were used, utilizing various objects of different sizes and shapes, and familiar and unfamiliar conspecifics. The objects in the EE cages changed every day and previously-seen objects were returned to the enrichment cage on the fifth day. Seventeen enrichment sessions of this nature occurred in total. The control group was handled in the same way as the enriched group except they were returned to their home cage instead of experiencing EE.

Object-in-Place Task and Data Analysis

Rats were tested in a 1-m^2 open field constructed of particle board surrounded by a white curtain to which an assortment of visual cues was pinned. The room in which testing took place had a low level of illumination and was sound insulated. Each testing session included an acclimation trial, two delay intervals (during which the subject was returned to the home cage), and two test trials (see Figure 4). Time (s) in contact with each object was measured during each trial. In order to be included in analysis, a subject needed to investigate each set of objects and explore all objects for at least 10 s in the sample phase. In addition, each subject was required to investigate each set of objects and explore all objects for a total of 10 s in the test phase to be included in analysis. Testing took place on PND 36 (OiP 1) and on PND 50 (OiP 2).

During the acclimation trial, the subject was placed in the center of the open field and allowed to investigate the four different, distinct objects, located in each of the four corners, for 3 min. Following a delay of 15 min from the acclimation trial, the subject was again placed in the open field and allowed to investigate the altered environment (Trial 2). For this trial, two objects exchanged positions. Following a delay of 60 min from the acclimation trial, the subject was returned to the open field for a final time, and was allowed to explore

the altered environment for 5 min, of which only the first 3 min of behavioral data was collected (Trial 3). The longer trial time was to allow for neural activation from the act of completing the task. For this trial, the remaining two objects exchanged positions (Figure 4).

Time investigating the objects in the field was of interest and was measured as time spent in direct contact with each of the four objects. For the test trials, the contact time for each of the objects was summed to produce a total contact time with objects. Then, contact time for the switched objects was summed and a proportion of contact time at the switched object relative to total contact time with the objects was calculated. This proportion was the dependent variable of interest. For further analysis, all contact times were measured by multiple observers, and all trials were videotaped.

Histology

Following Trial 3, four EE subjects (2 male, 2 female) were sacrificed immediately. All other subjects (N=28) experienced 60 to 90 min of quiet and dark followed by an injection with a lethal dose of sodium pentobarbital (100 mg/kg b.w., ip). Upon the absence of corneal and tail reflex, each subject was perfused intracardially with phosphate buffered saline (PBS) followed by 4% paraformaldehyde in 10 mM phosphate buffer (PB). The brain was dissected out of the cranium and post fixed in 10% sucrose in 4% paraformaldehyde in PB for 5 days at 4 °C and then transferred to PB for storage at 4 °C.

Sagittal sections were taken at 50 μm from one hemisphere and representative sections were processed using immunohistochemistry (ihc) to visualize the neural activity marker c-fos in neurons. Floating sections were rinsed in PBS (2 x 5 min) and incubated in 1% hydrogen peroxide for 15 min. Sections were rinsed again in PBS (2 x 5 min) and incubated in 15% goat serum in 0.2% Triton-X for 60 min. Sections were then placed in rat

anti-c-fos made in rabbit (Santa Cruz, sc-52) for 40 h at 4 °C. Sections were rinsed in PBS (6 x 10 min) and incubated in biotinylated goat anti-rabbit secondary antibody (Vector) for 60 min, and rinsed in PBS again (3 x 10 min). Sections were then exposed to a peroxidase-labeled avidin-biotin complex for 1 h (Vector) and rinsed in PBS (2 x 10 min). Finally, sections were exposed to an enzyme substrate (VIP, Vector) for at least 2 min.

Following the ihc procedure, sections were mounted onto gel-coated slides and allowed to air dry. The sections were dehydrated in graded ethanols, cleared with toluene, and cover-slipped with Permount (Fisher). Alternate sections were processed for Nissl staining with thionin to see cytoarchitecture.

Microscopy and data analysis. Analyses of sections were made using a Nikon Eclipse microscope, a PixeLink digital camera, and stereological techniques. An atlas of the rat brain (Pellegrino, Pellegrino, & Cushman, 1969) was used to identify areas of interest, specifically the DG and CA1 regions of the hippocampus (Figure 1).

The levels of activation were compared between enriched (EE) and control (no-EE) conditions in these brain regions. Initially, sections for c-fos positive (fos+) neuron density counts were identified by scanning sections using Plan 4 and 10 objectives. Neuron densities were counted using the Plan 10 objective and a 1024 x 768 pixel image. A counting frame was placed over the image and neurons marked for darkness (light, medium, or dark), which was used to indicate the extent of c-fos protein present and thus the extent of neural activation, in each sampling frame. Neurons marked as medium and dark were used for analyses. Objects that could not be identified were not counted. This yielded 3 (270 μm x 270 μm) samples per DG and CA1 section from each brain. Then, sample counts were averaged across sections to produce one value for each brain for each hippocampal region.

Results

Object-in-Place Task

The interaction between EE (EE or no-EE) and delay (15 or 60 min) on performance of the OiP task was of interest at both PND testing periods. At PND 36, the hypothesis that there would be an interaction between EE and delay was supported (see Figure 5). There was a significant interaction between EE and delay ($F(1,23)= 10.26$, $p< .0040$), with EE animals spending about half of their time exploring the rearranged objects at 15 min ($M = 0.53$, $SD = 0.10$) while no-EE animals spent a greater proportion of time with the rearranged objects at 15 min ($M = 0.65$, $SD= 0.16$), $t(23) = 2.05$, $p < .0519$. At the 60 min delay, EE animals again spent an equal proportion of time with the rearranged objects of interest ($M= 0.57$, $SD= 0.15$) while no-EE animals spent less time exploring these objects ($M = 0.39$, $SD = 0.23$), $t(23) = 2.86$, $p < .0089$. There was an overall effect of EE on the time spent exploring all objects, with EE animals spending, on average, a greater amount of time (s) with the objects ($M = 45.7$, $SD = 12.3$) than no-EE animals ($M = 31.7$, $SD = 13.1$), $F(1,26) = 15.71$, $p < .0005$ (Table 1).

At PND 50, the hypothesis that there would be an interaction between EE and delay was not supported (see Figure 5). The interaction between EE and delay on proportion of time with the rearranged objects was not significant ($F(1,19) = 1.03$, $p < .3240$), with EE animals spending about half of their time exploring the objects of interest at 15 min ($M= 0.62$, $SD= 0.22$). While no-EE animals spent a greater proportion of time with the rearranged objects ($M = 0.71$, $SD = 0.21$) in comparison to EE animals, the difference was not statistically significant, $t(19) = 1.29$, $p < .2125$. At the 60 min delay, EE animals spent less time with the rearranged objects than in the previous trial($M = 0.36$, $SD = 0.27$), and roughly

the same amount of time with these objects compared to no-EE animals, (M = 0.40, SD = 0.23) however this difference was not significant, $t(19) = 0.60$, $p < .5556$. Again, there was an effect of EE on overall time spent with all objects, with EE animals spending less time on average with the objects (M = 28.9, SD = 15.1) as compared to no-EE animals (M = 41.5, SD = 21.1), $F(1,25) = 4.78$, $p <.0383$ (Table 2).

Histology

The hypothesis that EE animals would show an increase in CA1 and a decrease in DG neuron activation relative to no-EE animals as indicated by counts of fos+ neurons was not supported (see Table 3). Rather, EE animals showed significantly less (-24.4%) neural activation in CA1 compared to no-EE animals, as indicated by count of fos+ neurons, $F(1, 21) = 5.17$, $p < .0336$. In the DG, brains of EE animals showed similar activation (+6.8%) as observed in brains of no-EE animals, $F(1, 21) = 0.22$, $p < .6419$ (see Figure 6).

Discussion

The current study examined the effects of EE on performance in a novelty preference task (i.e., OiP) in adolescent animals. Neural activation in relevant brain regions (i.e., DG and CA1) was also investigated. Thus, the behavioral and neural consequences of an enriching experience were examined in a complex object preference task designed to assess preference for novelty.

On PND 36, there was a significant interaction of EE and delay. Differences between groups (EE and no-EE) were seen at both 15 min and 60 min delays. Relative to no-EE animals, EE animals spent less time exploring the rearranged objects at the 15 min delay and more time with these objects at the 60 min delay. However, at both delays EE animals spent roughly the same proportion of time with the rearranged and familiar objects. Thus, EE

animals did not show a preference for novelty on PND 36. In addition, there was a main effect of EE on total exploration time of all objects, with EE animals spending more time exploring all objects at both delays, relative to no-EE animals.

Contrary to adult EE literature, which has shown that EE promotes increased novelty preference in both NLP and OiP tasks (e.g., Dix & Aggleton, 1999; Meehan & Mench, 2002), these results suggest that at PND 36, EE promotes adaptation to the rearrangement of the environment in adolescent animals. In addition, the total exploration times indicate that EE also changes exploration strategies of adolescent rats. Thus, EE animals investigate the entire environment and the objects within that environment without showing a preference for novelty. This is perhaps not surprising when considering that EE provides myriad opportunity for animals to practice exploration of novel, rearranged environments (Forgays & Forgays, 1952; Lynn & Brown, 2009).

Similar to results seen in Stansfield & Kirstein (2005) which showed a short latency to approach novelty in adolescent animals, the no-EE adolescent animals in this study demonstrated novelty preference very early (at 15 min). These results show that, in general, adolescent animals perform differently than adults on this novelty preference task, as no-EE animals showed a preference for novelty at 15 min, but not at 60 min. Cost et al. (2014) and Dix and Aggleton (1999) have demonstrated that novelty preference in adults (PND 75 and later) does not occur until 60 min.

On PND 50, no significant interaction between EE and delay was found. Again, no-EE animals spent more time with novelty at 15 min, however this was not significant. In addition, a main effect of EE was seen on total exploration time of all objects at both delays, with EE animals exploring the objects less than no-EE animals.

The general trends of PND 50 results were similar to those seen at PND 36, therefore, it is possible that, because the variability at PND 50 was higher than at PND 36, a statistically significant difference between the two groups was not seen. In addition, OiP 2 is the second time that animals have done the task. While it would not be expected for the animals to remember the objects in the field, performance might be different from OiP 1 as a result of the repeat testing design.

Lastly, the total exploration time results indicate an age-related effect on exploration and investigation strategies. Lynn & Brown (2009) showed that late adolescent rats (PND 47-59) explore more in an open field than early adolescent rats (PND 21-33). Such age-related changes in exploration strategies may explain the results at PND 50, as well, with EE animals exploring the open field (and not the objects within the field) more than at PND 36. Total exploration time (of the entire field) was not investigated in this experiment, but could be used to elucidate these results in the future.

CA1 and DG have been shown to respond divergently to novelty, with CA1 decreasing neural activation in novel environments and DG increasing (Nitz & McNaughton, 2004). It was hypothesized that, as a result of adaptation to novelty, CA1 interneuron activation would increase (i.e., firing in response to familiarity) in EE animals relative to no-EE animals. However, significant decrease in fos+ neurons in the CA1 of EE relative to no-EE animals was found, while there was no difference in DG activation between groups.

The DG is the input of the hippocampus, while the CA1 is the output of the hippocampus. No difference between groups in DG activation indicates that information about the novel environment arrangement is recognized by both EE and no-EE groups. A decrease in CA1 activation in EE brains relative to no-EE brains indicates that this

information is not all being consolidated. However, it is possible that as a result of EE, which provides opportunities to practice integrating novel environment information, the copious amounts of information about the environment is being pre-processed and some of that information excluded prior to arriving at the CA1. This indicates that EE brains are also adapted to novel environment rearrangement early on in the hippocampal pathway.

Thus, it can be concluded that the effect of EE happens early, both in age and in the brain pathway investigated. An interaction of delay and EE was only seen at PND 36, and histology results indicate that pre-processing of information occurs prior to reaching the CA1 in EE brains. It remains to be determined, however, if this is a result of re-testing. This may be elucidated by conducting a follow-up study that includes another experimental group that receives 17 EE sessions, yet is only tested once, at PND 50.

It may be also be important to note that because the animals were sacrificed on PND 50 (when there were no significant differences between groups behaviorally) the neural activation seen may not accurately reflect the behavioral results seen at PND 36. As such, it remains important to investigate neural activation at PND 36.

Neural results indicate that EE animals recognize novelty well, while also exhibiting pre-processing of novel environment information. Behaviorally EE animals do not show a preference to novelty (at least at PND 36). Thus, EE promotes adaptation to novelty in younger but not older adolescent animals, both in brain and behavior. These results indicate that adolescence is, in fact, a critical period for EE, and that EE that takes place earlier in adolescence is most effective at reducing risk-taking behaviors, such as novelty-seeking.

References

Ali, A. E. A., Wilson Y. M., & Murphy M. (2009). A single exposure to an enriched environment stimulates the activation of discrete neuronal populations in the brain of the fos-tau-lacZ mouse. *Neurobiology of Learning and Memory, 92*(3), 381-90. doi: 10.1251/bpo128

Andersen, P., Bliss, T.V.P., & Skrede, K.K. (1971). Lamellar organization of hippocampal excitatory pathways. *Experimental Brain Research, 13,* 222–238. doi: 10.1007/BF00234087

Barker, G. & Warburton, E. (2011). When is the hippocampus involved in recognition memory? *Journal of Neuroscience, 31*,10721-10731. doi: 10.1523/JNEUROSCI.6413-10.2011

Barker, G., Bird, F., Alexander, V., and Warburton, E. (2007). Recognition memory for objects, place, and temporal order: A disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *Journal of Neuroscience, 27,*2948-2857. doi: 10.1523/JNEUROSCI.5289-06.2007

Barker, G., Warburton, E. (2009). Critical role of the cholinergic system for object-in-place associative recognition memory. *Learning and Memory, 16*, 8-11. doi: 10.1101/lm.1121309

Barnea, A., and Nottebohm, F. (1996). Recruitment and replacement of hippocampal neurons in young and adult chickadees: An addition to the theory of hippocampal learning. *Proceeding of the National Academy of Science, 93,* 714-718.

Barnea, A., Mishal, A., and Nottebohm, F. (2006). Social and spatial changes induce multiple survival regimes for new neurons in two regions of the adult brain: An anatomical

representation of time? *Behavioral Brain Research, 167,* 63-74. doi: 10.1016/j.bbr.2005.08.018

Bennett, E.L., Rosenzweig, M.R., Morimoto, H., & Herbert, M. (1979). Maze training alters brain weights and cortical RNA/DNA ratios. *Behavioral and Neural Biology, 26,* 1-22.

Bennett, J. C., McRae, P.A., Levy, L.J., and Frick, K.M. (2006). Long-term continuous, but not daily, environmental enrichment reduces spatial memory decline in aged male mice. *Neurobiology of Learning and Memory, 85*, 139-152. doi: 10.1016/j.nlm.2005.09.003

Blakemore, S.J., & Choudhury, S. (2006). Development of the adolescent brain: Implications for executive function and social cognition. *Journal of Child Psychology & Psychiatry, 47,* 296-312. doi: 10.1111/j.1469-7610.2006.01611.x

Bouchon, R., & Will, B. (1982). Effects of early enriched and restricted environments on the exploratory and locomotor activity of dwarf mice. *Behavioral Neural Biology, 35*(2), 174-86.

Cain, M. E., Green, T. A., & Bardo, M. T. (2006). Environmental enrichment decreases responding for visual novelty. *Behavioral Processes, 73*(3), 360-6. doi: 10.1016/j.beproc.2006.08.007

Candland, D.K., & Cambell, B.A. (1962). Development of fear in the rat as measured by behavior in the open field. *Journal of Comparative & Physiological Psychology, 55*, 593-596.

Cheng, S., & Frank, L. M. (2008). New experiences enhance coordinated neural activity in the hippocampus. *Neuron, 57*(2), 303-13. doi: 10.1016/j.neuron.2007.11.035

Cobb, D. E., and Zrull, M.C. (2014). Environmental enrichment during adolescence reduces affinity for novelty in young adult rats. *Society for Neuroscience Abstracts, 180.07* [available online]. Paper presented at the Society for Neuroscience 44th Annual Meeting, Washington, DC.

Cost, K. T., Lobell, T. D., Williams-Yee, Z. N., Henderson, S., & Dohanich, G. (2014). The effects of pregnancy, lactation, and primiparity on object-in-place memory of female rats. *Hormones and Behavior, 65*(1), 32-9. doi: 10.1016/j.yhbeh.2013.10.012

Dhanushkodi, A., Bindu, B., Raju, T. R., & Kutty, B. M. (2007). Exposure to enriched environment improves spatial learning performance and enhances cell density but not choline acetyltransferase activity in the hippocampus of ventral subicular-lesioned rats. *Neuroscience, 135*(2), 395-402. doi: 10.1037/0735-7044.121.3.491

Diamond, M. C., Ingham, C. A., Johnson, R. E., Bennett, E. L., & Rosenzweig, M. R. (1976). Effects of environment on morphology of rat cerebral cortex and hippocampus. *Journal of Neurobiology, 7,* 75–85. doi: 10.1002/neu.480070108

Dix, S. L., & Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behavioral Brain Research, 99,* 191-200. doi: 10.1016/S0166-4328(98)00079-5

Fernandez-Teruel, A., Driscoll, P., Gil, L., Aguilar, R., Tobena, A., & Escorihuela, R. M. (2002). Enduring effects of environmental enrichment on novelty seeking, saccharin and ethanol intake in two rat lines (RHA/Verh and RLA/Verh) differing in incentive-seeking behavior. *Pharmacology Biochemistry and Behavior, 73*(1), 225-31. doi: 10.1016/S0091-3057(02)00784-0

Forgays, D. G., & Forgays, J. W. (1951). The nature of the effect of free-environmental experience in the rat. *Journal of Comparative Physiolgical Psychology, 45*(4), 322-8.

Foster, T. C., & Dumas, T. C. (2001). Mechanism for increased hippocampal synaptic strength following differential experience. *Journal of Neurophysiology, 85*, 1377–1383.

Green, E. J., McNaughton, B. L., & Barnes, C. A. (1990). Exploration-dependent modulation of evoked responses in fascia dentata: Dissociation of motor, EEG, and sensory factors and evidence for a synaptic efficacy change. *Journal of Neuroscience, 10*, 1455–1471.

Hymovitch, B. (1952). The effects of experimental variations on problem solving in the rat. *Journal of Comparative Physiological Psychology, 45*(4), 313-21.

Kempermann, G., & Gage, F. H. (1999). Experience-dependent regulation of adult hippocampal neurogenesis: Effects of long-term stimulation and stimulus withdrawal. *Hippocampus, 9,* 321–332. doi: 10.1002/(SICI)1098-1063(1999)9:3<321::AID-HIPO11>3.0.CO;2-C

Kempermann, G., Kuhn, H. G., & Gage, F. H. (1997). More hippocampal neurons in adult mice living in an enriched environment. *Nature, 386,* 493–495. doi: 10.1038/386493a0

Lee, I., Hunsaker, M.R., & Kesner, R.P. (2005). The role of hippocampal subregions in detecting spatial novelty. *Behavioral Neuroscience 119,* 145–153. doi: 10.1037/0735-7044.119.1.145

Lynn, D. A., & Brown, G. R. (2009). The ontogeny of exploratory behavior in male and female adolescent rats (*Rattus norvegicus)*. *Developmental Psychobiology, 51*(6), 513-20. doi: 10.1002/dev.20386

Meehan, C. L., & Mench, J. A. (2002). Environmental enrichment affects the fear and exploratory responses to novelty of young Amazon parrots. *Applied Animal Behavior Science, 79*, 75-88. doi: http://dx.doi.org/10.1016/S0168-1591(02)00118-1

Meeter, M., Murre, J.M., & Talamini, L.M. (2004). Mode shifting between storage and recall based on novelty detection in oscillating hippocampal circuits. *Hippocampus, 14,* 722–741. doi: 10.1002/hipo.10214

Moser, E. I., Moser, M. B., & Andersen, P. (1994). Potentiation of dentate synapses initiated by exploratory learning in rats: Dissociation from brain temperature, motor activity, and arousal. *Learning and Memory, 1*, 55–73. doi: 10.1101/lm.1.1.55

Nitz, D. & McNaugton, B. (2004). Differential modulation of CA1 and dentate gyrus interneurons during exploration of novel environments. *Journal of Neurophysiology, 91*, 863-72. doi: 10.1152/jn.00614.2003

Pellegrino, L. J., Pellegrino, A. S., & Cushman, A. J. (1969). *A stereotaxic atlas of the rat brain.* New York: Plenum Press.

O'Keefe, J. & Dostrovsky, J. (1971). The hippocampus as a spatial map: Preliminary evidence from unit activity in the freely-moving rat. *Brain Res, 34*(1), 171-5. doi: 10.1016/0006-8993(71)90358-1

O'Keefe, J., & Nadel, L. (1978). *The Hippocampus as a Cognitive Map*. London:Oxford University Press.

Olsson, A., & Dahlborn K. (2002). Improving housing conditions for laboratory mice: A review of "environmental enrichment." *Laboratory Animals 36:*243–270. doi: 10.1258/002367702320162379

Rampon, C., Tang, Y. P., Goodhouse, J., Shimizu, E., Kyin, M., & Tsien, J. Z. (2000). Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. *Nature Neuroscience, 3*, 238–244. doi: 10.1038/72945

Rosenzweig, M. R. (2003). Effects of differential experience on the brain and behavior. *Developmental Neuropsychology, 24,* 523–540. doi: 10.1080/87565641.2003.9651909

Rosenzweig, M. R., & Bennett, E. L. (1996). Psychobiology of plasticity: Effects of training and experience on brain and behavior. *Behavioral Brain Research, 78,* 57–65. doi: 10.1016/0166-4328(95)00216-2

Sengupta, P. (2013). The laboratory rat: Relating its age with human's. *International Journal of Preventative Medicine, 4*, 624-630.

Simpson, J., & Kelly, J. P. (2011). The impact of environmental enrichment in laboratory rats—Behavioural and neurochemical aspects. *Behavioral Brain Research, 222*(1), 246-64. doi: 10.1016/j.bbr.2011.04.002

Spear, L.P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Reviews, 24,* 417-463. doi: 10.1016/S0149-7634(00)00014-2

Stansfield, K. H., & Kirstein, C. L. (2005). Effects of novelty on behavior in the adolescent and adult rat. *Developmental Psychobiolpgy, 48*(1), 10-15. doi: 10.1002/dev.20127

Tirelli, E., Laviola, G., & Adriani, W. (2003). Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. *Neuroscience and Biobehavioral Reviews, 27*, 163-178. doi: 10.1016/S0149-7634(03)00018-6

van Praag, H., Kempermann, G., & Gage, F. H. (2000). Neural consequences of environmental enrichment. *Nature Reviews Neuroscience, 1*(3), 191-8. doi: 10.1038/35044558

VanElzakker, M., Fevurly, R. D., Breindel, T., & Spencer, R. L. (2008). Environmental novelty is associated with a selective increase in Fos expression in the output elements of the hippocampal formation and the perirhinal cortex. *Learning and Memory, 15*(12), 899-908. doi: 10.1101/lm.1196508

Will, B., Toniolo, G., Kelche, C., Pallage, V., Deluzarche, F., & Misslin, R. (1986). The effects of postoperative physical environment on novelty seeking behavior and maze learning in rats with hippocampal lesions. *Behavioral Brain Research, 19*, 233-240.

Würbel, H., Stauffacher, M., & von Holst, D. (1996). Stereotypies in laboratory mice—Quantitative and qualitative description of the ontogeny of wire-gnawing and jumping in ICR and ICR nu-mice. *Ethology, 102,* 371-385.

Table 1.

Time (seconds) Spent in Contact with All Objects during Testing Period on PND 36

Delay	Group	*N*	*M*	*SD*
15 min	EE	16	42.4*	10.1
	No-EE	14	31.6*	14.9
60 min	EE	15	49.3**	13.6
	No-EE	12	31.8**	11.3

Note. The significant difference is indicated (*$p < .03$, **$p < .002$). Abbreviations: EE, environmental enrichment; No-EE, no EE; postnatal day, PND.

Table 2.

Time (seconds) Spent in Contact with All Objects during Testing Period on PND 50

Delay	Group	*N*	*M*	*SD*
15 min	EE	13	32.5*	15.9
	No-EE	12	44.6*	20.5
60 min	EE	14	25.6*	14.2
	No-EE	13	38.7*	22.2

Note. The significant difference is indicated (*$p < .04$). Abbreviations: EE, environmental enrichment; No-EE, no EE; postnatal day, PND.

Table 3.

Mean Number of Activated Neurons in Hippocampal Regions for Enriched and Control (not Enriched) Animals.

	CA1			DG		
Group	*N*	*M*	*SD*	*N*	*M*	*SD*
EE	13	92.3*	33.6	13	132.3	46.5
No-EE	10	122.1*	27.5	10	123.9	35.8

Note. The significant difference is indicated (*$p < .05$). Abbreviations: environmental enrichment, EE; No-EE, no EE; *cornu ammonis* 1, CA1; dentate gyrus, DG.

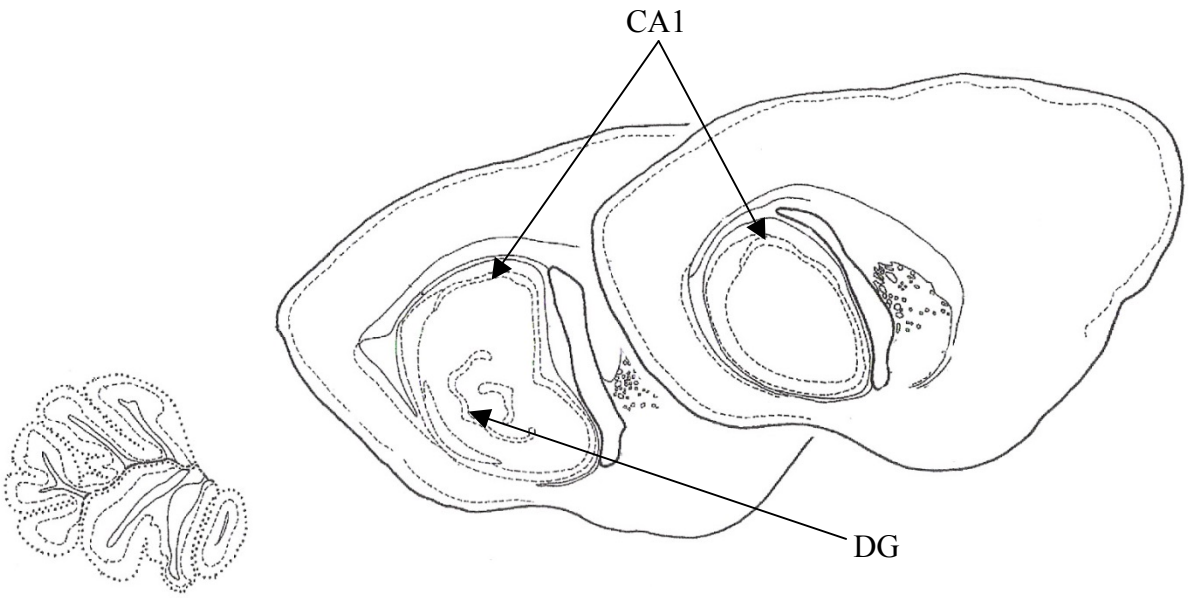


Figure 1. Examples of sampled locations of CA1 and DG are indicated by arrows.

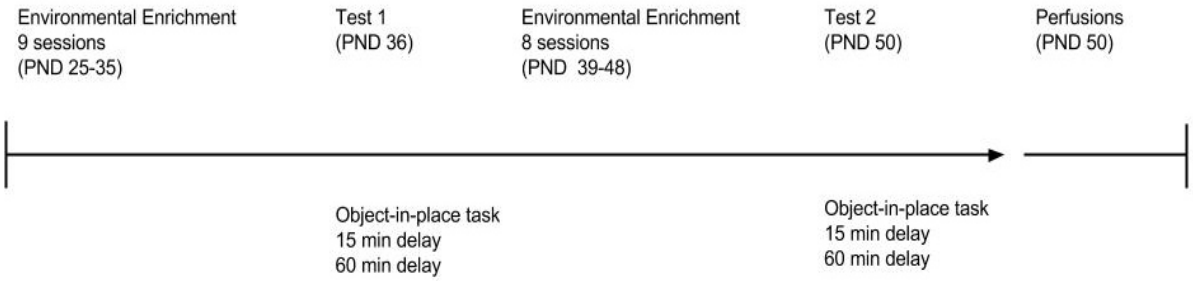


Figure 2. Nine sessions of EE took place (PND 25-35) prior to OiP 1. Eight additional sessions of EE took place (PND 39-48), followed by OiP 2 and perfusion.

Figure 3. The picture shows an example of an enrichment cage with one arrangement of objects. EE cages were multi-level boxes and contained objects of different shape, color, and texture, in addition to same sex familiar and unfamiliar conspecifics.

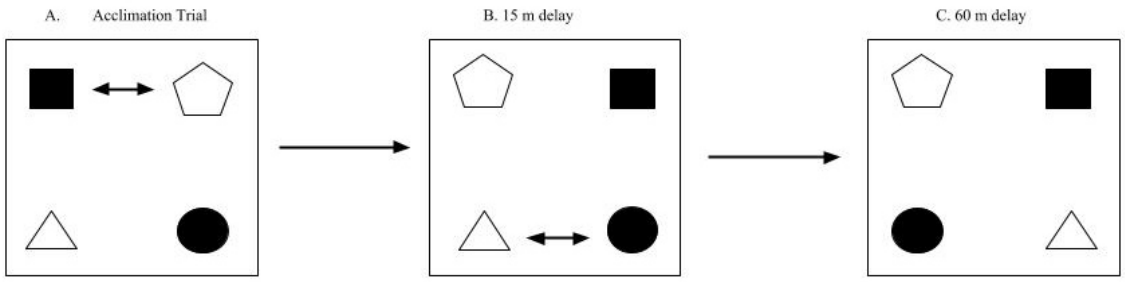
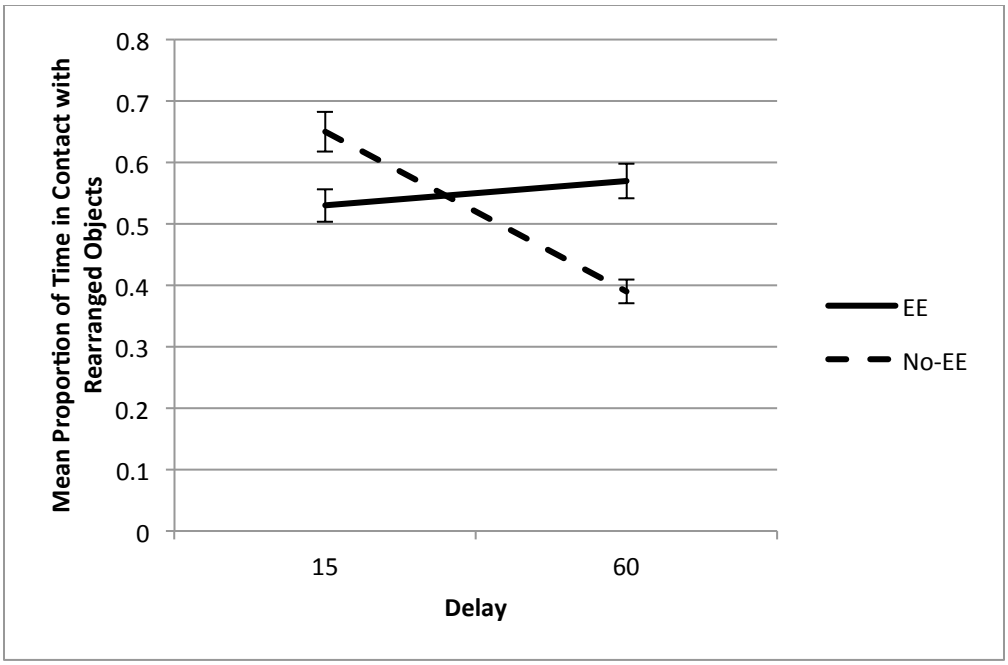


Figure 4. (A) Rats investigated objects for 3 min during the acclimation phase. (B) After a delay of 15 min, rats investigated the rearrangement of objects for 3 min. (C) After a delay of 60 min, rats investigated the final rearrangement of objects for 5 min, of which only the first 3 min of behavior data was collected.

A.



B.

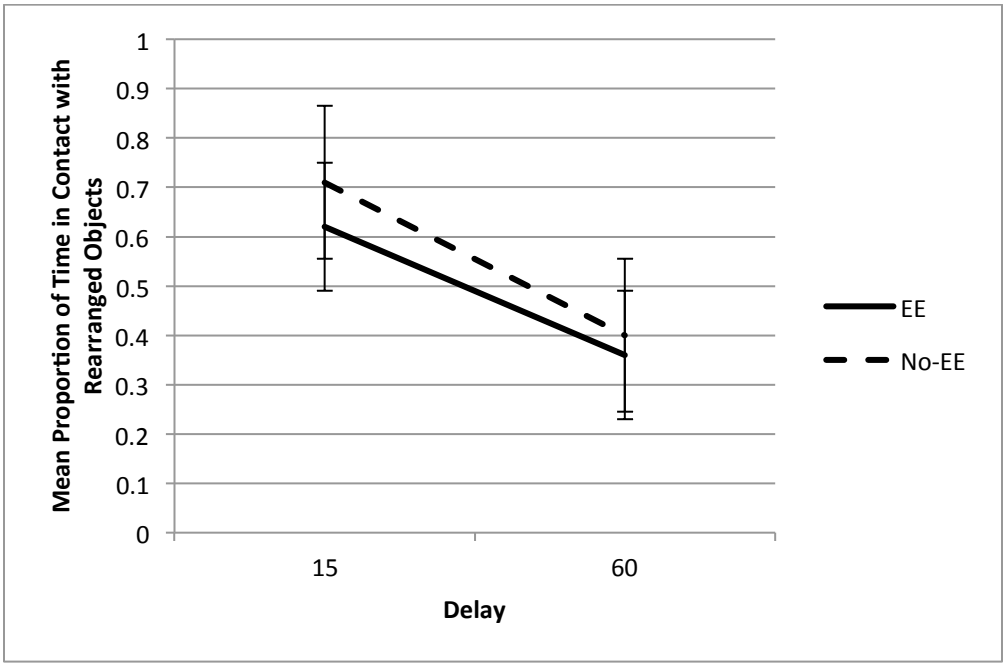


Figure 5. (A) At PND 36, a significant interaction between EE and delay was seen. (B) At PND 50, no significant interaction between EE and delay was seen.

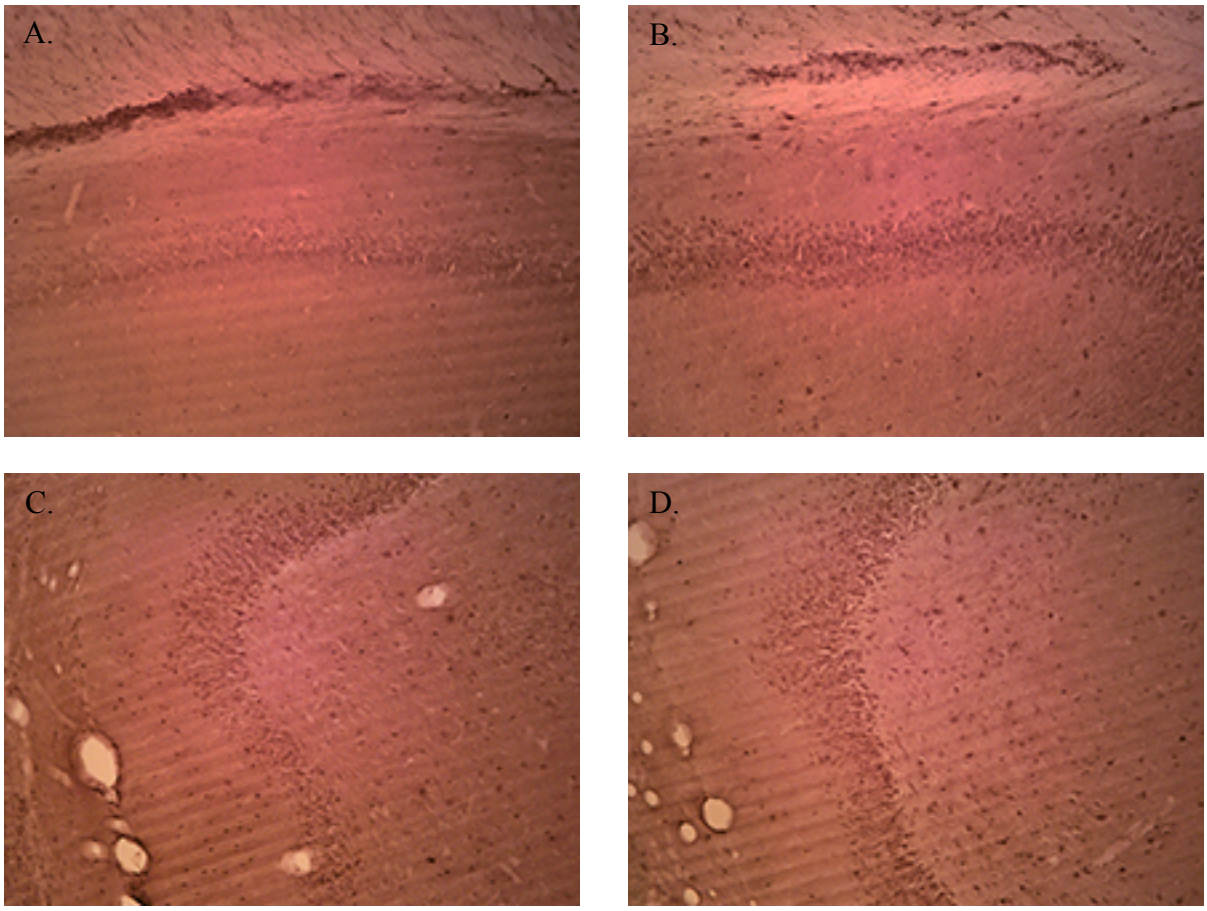


Figure 6. (A) CA1 region of the hippocampus in EE animals. (B) CA1 region of the hippocampus in no-EE animals. EE animals showed a 24.4% reduction in fos+ cells in this region compared to no-EE animals. (C) DG region of the hippocampus in EE animals. (D) DG region of the hippocampus in no-EE animals. No significant difference was seen between groups in this region.